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DIURNAL ACID METABOLISM IN *ISOETES HOWELLII* FROM A TEMPORARY POOL AND A PERMANENT LAKE¹

JON E. KEELEY, R. PATRICK MATHEWS, AND
CINDY MILLER WALKER

Biology Department, Occidental College, Los Angeles, California 90041

ABSTRACT

Water chemistry and titratable acidity and malic acid levels in *Isoetes howellii* leaves were sampled every 6 hr from plants in a seasonal pool and an oligotrophic lake. Plants in the seasonal pool showed a diurnal fluctuation of ~ 300 μeq titratable acidity g^{-1} fresh wt; daytime deacidification was 75% complete by noon and nighttime acidification was 45% complete by midnight. Late in the season after the pool had dried, emergent leaves showed only a very weak tendency to accumulate acid at night. Plants from the oligotrophic lake had a diurnal change of ~ 100 μeq g^{-1} fresh wt, daytime deacidification was only 45% complete by noon but nighttime acidification was 80% complete by midnight. Water characteristics were distinctly different between these two systems. In the seasonal pool there were marked diurnal changes in temperature, pH, oxygen and carbon dioxide. Free- CO_2 levels were an order of magnitude greater in the early morning than in the late afternoon. In contrast, the conditions in the oligotrophic lake showed no marked diurnal fluctuation, though total inorganic carbon levels were extremely low relative to other aquatic systems.

CRASSULACEAN ACID METABOLISM (CAM) has been demonstrated in the submerged aquatic *Isoetes howellii* Engelmann (Isoetaceae) (Keeley, 1981a; Keeley and Bowes, 1982). Leaves, but not corms, are capable of substantial net CO_2 uptake in the dark which is fixed into malic acid and other organic acids. PEP carboxylase activity is sufficient to account for rates of dark CO_2 fixation and rates of overnight malic acid accumulation. Daytime deacidification produces a diurnal fluctuation of 100–300 μeq titratable acidity per g fresh wt. This carbon fixation pathway is largely turned off in the light and dark-fixed carbon appears to move from organic acids into phosphoglycerate and other C_3 -pathway products.

In the prototype terrestrial CAM plant, diurnal acid metabolism is coupled with a diurnal pattern of low stomatal resistance at night and high resistance during the day. In these "Super-CAM" plants (Kluge and Ting, 1978) the bulk of the carbon assimilation is derived from nighttime CO_2 fixation. In other terrestrial succulents exhibiting crassulacean acid metabolism, both light and dark CO_2 uptake contribute to net carbon assimilation.

Isoetes howellii possess stomata though, as

in other aquatics (Sculthorpe, 1967), they are apparently nonfunctional due to a wax occlusion of the aperture (Keeley, 1981b). The presence of diurnal acid metabolism in aquatic *Isoetes* lacking stomata (Keeley, 1982) is evidence that stomata are not involved in this phenomenon.

Isoetes howellii (and *Isoetes storkii*, Keeley et al., 1981) is capable of net CO_2 uptake in both the light and dark and under similar carbon conditions CO_2 uptake in the light is substantially greater than dark CO_2 uptake (Keeley, 1981a; Keeley and Bowes, 1982). Previously it was hypothesized that diurnal fluctuations in CO_2 availability in the water could limit daytime CO_2 uptake and put a premium on nighttime CO_2 uptake. Some support for this hypothesis has been reported for a southern California vernal pool (Keeley, 1981b; Keeley, 1983).

The purpose of this study was to investigate the dynamics of the acidification/deacidification cycle in *Isoetes howellii* leaves and concomitant changes in water chemistry for two habitats representing a range of conditions.

METHODS—Study sites—*Isoetes howellii* is commonly found in the unique California Vernal Pool Ephemeral Community (Thorne, 1976) and has been investigated in some detail there. However, it is found throughout the western United States in various aquatic situations. The *Isoetes howellii* habitats investigated here were a temporary pool located near

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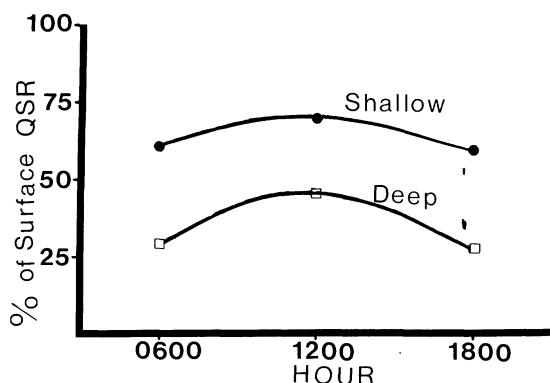


Fig. 1. Percent of surface quantum solar flux at shallow (5–10 cm) and deep (15–20 cm) depths in Mather Pool on 29 May 1981.

the Carnegie Field Station at Mather (1,375 m), Tuolumne Co., CA and a permanent lake, Woahink Lake (10 m), Lane Co., OR. The Mather pool is seasonal, though it is located at a higher elevation than “Vernal Pools” and has none of the ephemeral annuals characteristic of the Vernal Pool Community. Woahink Lake is a sparsely vegetated oligotrophic lake.

Pool parameters—Photon flux density was measured with a LI Cor LI-188B integrating meter with a LI-190SB quantum sensor at, and perpendicular to, the water surface and a LI-192SB underwater quantum sensor at the underwater level of the plants. Specific con-

ductance was measured with a YSI-33 conductivity meter at 25 C. Dissolved oxygen was determined with a YSI-57 oxygen meter and YSI-5700 polarographic sensor. Carbon dioxide was determined, according to APHA (1976) methods, on water samples kept on ice until assayed (usually within 1 hr) by titrating to pH 8.3 with CO₂-free 0.0227 N NaOH at ambient temperature using a Photovolt 126-A meter. Alkalinity was determined by titrating with CO₂-free 0.02 N H₂SO₄. A pH 4.5 endpoint was used for total alkalinity on the Mather samples, but because of very low alkalinity levels in Woahink Lake, a pH 5.1 endpoint was used, as recommended by APHA (1976).

Plant samples were kept on ice until extraction, usually within an hour. Leaf samples <0.5 g were washed, blotted dry and weighed on a Ohaus 300 electronic balance (run on car battery). Extracts were prepared by grinding in a Ten Broeck with 15.0 ml cold CO₂-free deionized water and spun down with a desk top centrifuge (run on a gas powered generator). A 1.0-ml sample of supernatant was deproteinized with an equal volume of 0.6 N perchloric acid and returned to the lab and assayed spectrophotometrically for malic acid with an enzymatic end product assay (Gutmann and Wahlefeld, 1974). A 10.0-ml sample was titrated immediately after extraction with CO₂-free 0.01 N NaOH to pH 6.4 and pH 8.3 which represents the range of values used in studies of acid fluctuations in CAM plants.

TABLE 1. Quantum solar radiation at the water surface, physical and chemical characteristics of the water and titratable acidity (to pH 6.4) and malic acid levels in *Isoetes howellii* in shallow (S; 5–10 cm) and deep (D; 15–20 cm) depths of the seasonal pool at Mather, 29–30 May 1981. Specific conductance of the water was 73 $\mu\text{mhos cm}^{-1}$

Conditions	Hour									
	0600		1200		1800		2400		0600	
QSR ($\mu\text{E m}^{-2} \text{ s}^{-1}$)	40		1,860		125		0		30	
	S	D	S	D	S	D	S	D	S	D
WATER										
Temperature (C)	16	17	23	21	28	26	24	21	17	17
Oxygen (% saturation)	34	62	148	119	171	151	90	76	34	68
pH	6.6	6.8	6.8	6.8	7.6	7.1	6.9	6.8	6.5	6.7
Free-CO ₂ (mg l ⁻¹)	26.6	15.3	8.1	8.6	2.9	6.1	10.0	9.8	27.6	13.3
HCO ₃ ⁻ & CO ₃ ²⁻ (mg l ⁻¹) ^a	48.0	47.9	38.0	38.2	41.9	40.9	48.8	48.8	47.0	45.0
SUBMERGED LEAVES (Deep)										
Acidity ($\mu\text{eq g}^{-1}$ FW) ^b	297 ± 53		121 ± 23		49 ± 3		181 ± 18		339 ± 0	
Malic Acid ($\mu\text{mol g}^{-1}$ FW)	159 ± 26		67 ± 13		27 ± 2		100 ± 14		180 ± 1	
SUBMERGED LEAVES (Shallow)										
Acidity ($\mu\text{eq g}^{-1}$ FW)	355 ± 30		125 ± 31		48 ± 10		179 ± 59		327 ± 31	
Malic Acid ($\mu\text{mol g}^{-1}$ FW)	202 ± 41		70 ± 11		27 ± 6		126 ± 4		145 ± 25	

^a As CaCO₃.

^b $\bar{x} \pm \text{S.D.}$, $n = 2$, FW = fresh weight.

TABLE 2. Quantum solar radiation, temperature, and titratable acidity (to pH 6.4) and malic acid levels in *Isoetes howellii* growing in saturated soil with leaves exposed to the atmosphere, following the drying up of the Mather Pool, 29–30 June 1981

Conditions	Hour				
	0600	1200	1800	2400	0600
QSR ($\mu\text{E m}^{-2} \text{s}^{-1}$)	30	1,760	540	0	40
AIR TEMPERATURE (C)	13	32	29	13	11
EMERGENT LEAVES					
Acidity ($\mu\text{eq g}^{-1} \text{FW}$) ^a	29 \pm 1	25 \pm 1	0 \pm 0	13 \pm 2	17 \pm 0
Malic Acid ($\mu\text{mol g}^{-1} \text{FW}$)	43 \pm 4	27 \pm 1	24 \pm 9	33 \pm 3	52 \pm 1

^a $\bar{x} \pm \text{S.D.}$, $n = 2$, FW = fresh weight.

RESULTS—Mather Pool—The pool was first visited on 6 May 1981, at which time it was densely vegetated although *Isoetes howellii* was not present. On 29–30 May, *I. howellii* was present (though rare) and the physical and chemical characteristics of the pool and acidity changes in the leaves at that time are shown in Table 1. Quantum solar flux at the water surface is also shown in Table 1 and the reduction at two underwater levels is shown in Fig. 1. At a shallow depth (5–10 cm) noontime quantum solar flux was $\sim 70\%$ of surface levels. At a deep underwater level (15–20 cm) noontime quantum radiation was $\sim 45\%$ of surface level. During the day, shallow water became warmer, oxygen saturation was greater and free- CO_2 depletion greater than deep water. Diurnal changes in titratable acidity and malic acid in *I. howellii* leaves did not vary significantly between shallow and deep levels (Table 1). Titratable acidity measured at pH 6.4 accounted for 97–99% of the diurnal fluctuation measured at pH 8.3 (data not shown). The diurnal change in acidity was 250–300 $\mu\text{equivalents per g fresh}$

wt. Daytime deacidification was $\sim 75\%$ complete by noon and overnight acidification was $\sim 45\%$ complete by midnight.

By late June, standing water was no longer present though the soil was still moist. Emergent leaves showed an order of magnitude less acid change (Table 2) than previously sampled submerged leaves.

Woahink Lake—Unlike the Mather Pool this lake showed little diurnal change in temperature, oxygen saturation, pH or free- CO_2 (Table 3). Total carbon in the water was substantially less than at Mather. Most *Isoetes* plants were growing in shallow water where the tips of the leaves were 5 cm below the surface. Noontime quantum solar flux at this level was $\sim 80\%$ of surface level. The diurnal change in titratable acidity at pH 6.4 accounted for 94–95% of the pH 8.3 change and was $\frac{1}{3}$ that observed in *I. howellii* leaves at Mather. The dynamics of the acidification/deacidification cycle were distinctly different from those observed at Mather. Daytime deacidification was only $\sim 45\%$

TABLE 3. Quantum solar radiation at the water surface, physical and chemical characteristics of the water and titratable acidity (to pH 6.4) and malic acid levels in *Isoetes howellii* in the oligotrophic Woahink Lake, 31 July–1 August 1981. Specific conductance of the water was 71 $\mu\text{mhos cm}^{-1}$

Conditions	Hour				
	0600	1200	1800	2400	0600
QSR ($\mu\text{E m}^{-2} \text{s}^{-1}$)	30	1,730	855	0	10
WATER					
Temperature (C)	21	22	21	21	21
Oxygen (% saturation)	106	108	99	99	100
pH	6.4	6.4	6.4	6.4	6.1
Free- CO_2 (mg l^{-1})	8.2	7.9	7.7	7.5	12.1
HCO_3^- and CO_3^{2-} (mg l^{-1}) ^a	7.8	7.9	8.9	8.7	6.1
SUBMERGED LEAVES					
Acidity ($\mu\text{eq g}^{-1} \text{FW}$) ^b	138 \pm 5	79 \pm 15	11 \pm 3	31 \pm 3	114 \pm 2
Malic Acid ($\mu\text{mol g}^{-1} \text{FW}$)	87 \pm 6	63 \pm 9	26 \pm 2	80 \pm 30	69 \pm 13

^a As CaCO_3 .

^b $\bar{x} \pm \text{S.D.}$, $n = 2$, FW = fresh weight.

complete by noon whereas nighttime acidification was ~80% complete by midnight.

DISCUSSION—The diurnal fluctuation in acidity for *Isoetes howellii* leaves observed in the Mather Pool plants are the highest levels thus far observed for *Isoetes*. They are ~double the levels previously reported for southern California vernal pool plants (Keeley 1981a, b, 1983). Perhaps not surprisingly the free-CO₂ levels at night in the Mather Pool were ~double those observed in vernal pools (Keeley 1981b, 1983). A similar relationship holds for Woahink Lake where overnight free-CO₂ levels were 1/3 those observed in the Mather Pool and overnight acid accumulation was 1/3 that observed in *I. howellii* from the Mather Pool.

In comparing the Mather Pool with Woahink Lake it is clear that *I. howellii* can exist under distinctly different carbon regimes. In the seasonal pool there was an order of magnitude depletion in free-CO₂ during the day (Table 1). In contrast, Woahink Lake showed no diurnal flux in CO₂ level, though total carbon was at the low end for aquatic habitats, being five times below the level in the Mather Pool.

Previously it was hypothesized that crassulacean acid metabolism was selected for in *I. howellii* to provide an internal CO₂ source during the day when CO₂ became limiting to photosynthesis. The hypothesis is strongly supported by data from southern California Vernal Pools where free-CO₂ is often totally depleted by noon on sunny days. In those pools the high pH and low CO₂ conditions during the afternoon reduce CO₂ uptake orders of magnitude below morning CO₂ uptake rates (Keeley, 1983).

The extent of carbon limiting conditions in either the Mather Pool or Woahink Lake is unclear. In both situations the free-CO₂ as well as the total inorganic carbon levels are high relative to air levels. However, the diffusive resistance of water could easily produce carbon limiting conditions in the boundary layer around the leaf as demonstrated for various aquatic plants (Prins et al., 1980; Prins and Helder, 1981). This is particularly likely in the stagnant water conditions typical of both habitats studied here. If boundary layer effects are

important, the order of magnitude increase in free-CO₂ level overnight in the Mather Pool could put a premium on dark CO₂ uptake. In the oligotrophic Woahink Lake, low carbon levels day and night could put a premium on continuous light and dark CO₂ uptake.

The hypothesis that CAM has been selected for under carbon-limiting conditions is supported by the observation that as the plants become emergent from seasonal pools the pathway is largely lost (Table 2).

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